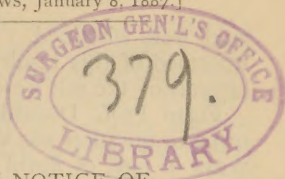


Marshall, (John) Comps of you

[From THE MEDICAL NEWS, January 8, 1887.]



A PRELIMINARY NOTICE OF
A CRYSTALLINE ACID IN URINE POSSESSING
MORE POWERFUL REDUCING PROPERTIES
THAN GLUCOSE.

BY JOHN MARSHALL, M.D.,
DEMONSTRATOR OF CHEMISTRY, UNIVERSITY OF PENNSYLVANIA.

In the early part of last November, Prof. Frank Donaldson, Sr., of Baltimore, sent for examination, to Prof. Tyson, of this city, a urine which contained a substance having strong reducing properties much resembling those possessed by glucose. After Prof. Tyson had finished his examination, he gave the remainder of the urine to Prof. Wormley, for further investigation. From Prof. Wormley it came to me. From the results obtained by the three independent observers it was concluded that the reducing substance was certainly not glucose. This conclusion was at once communicated to Prof. Donaldson by Prof. Tyson, and at the same time Prof. Tyson requested that a larger quantity of urine be sent to me for further examination. This larger quantity was kindly sent by Prof. Donaldson, and arrived at the University on November 17th, and the examination was immediately continued. A few days afterward (November 21) crystals of the lead salt of the

acid were obtained. Prof. Donaldson was at once informed of this result, and at the same time a few crystals of the salt were sent to him.

From Prof. Donaldson it was learned that the urine in question was voided by a man thirty-seven years of age, of florid complexion, and of average height and weight. His general health and nutrition have always been good. He has never had any muscular weakness or inordinate thirst; no emaciation, but instead a continued increase in weight; no excessive quantity of the secretions. He has always been temperate as to alcoholic stimulants. Since his seventeenth year he has been engaged in the lumber business, and at present is superintendent of a planing-mill, which position requires his visiting the mill two or three times daily.

The case is peculiarly interesting, because of the man's having repeatedly, during the past two and a half years, applied to the various life insurance companies represented in Baltimore for insurance, but each time suffering rejection because of the response of his urine to certain reagents used in testing for glucose, a response which naturally was considered to be due to glucose.

Upon the ingestion of certain substances, other substances appear in the urine, which have a reducing action upon alkaline copper solutions. When camphor is ingested, camphoglycuronic acid, $C_{16}H_{24}O_8$, appears in the urine. This breaks up into glycuronic acid, $C_6H_{10}O_7$, which has a strong reducing action. Chloroform in the urine also reduces alkaline copper solution. Chloral is converted into urochloralic acid, $C_8H_{13}Cl_3O_7$. Turpentine into turpenoglycuronic acid. Morphia forms a reducing substance. Phenol (carbolic acid) and benzol form hydrochinon, $C_6H_4(OH)_2$. Phenol and benzol are

also converted into oxyphenic acid (pyro-catechin), $C_6H_4(OH)_2$, which latter probably is identical with the substance described by Boedeker as alkapton. Tannic acid is excreted as gallic acid. All these products possess the property of reducing alkaline copper solution. Hydrochinon and oxyphenic acid in the presence of an alkali, and when exposed to the air absorb oxygen, and turn first green, then brown, and, finally, black.

It was learned that the person who voided the urine under examination never had occasion to use any of the above-named substances; and, therefore, one would hardly expect to find the products of their metamorphosis in the urine. It must not be forgotten, however, that oxyphenic acid has several times been found in normal urine.

The peculiar acid in question is contained in rather large quantity in this particular urine, nearly one gramme of the lead salt having been obtained from 100 c. c. of the urine. Its reducing power is greater than that of glucose; 0.6 c. c. of the undiluted urine was sufficient to reduce the cupric oxide in 10 c. c. of Fehling's solution, equivalent to 0.05 of glucose, or, expressed in glucose units, equivalent to 8.3 per cent. of glucose.

Some of the reactions of this urine, when considerably diluted with water or with normal urine, strikingly resemble reactions often noticed in this laboratory in urine considered and acknowledged to be free from glucose, especially in the reaction with diluted Fehling's solution. With the urine containing the acid, diluted either with water or with normal urine, and diluted Fehling's solution, a brownish and sometimes greenish coloration is produced, but no appreciable reduction of the cupric oxide is observed. A similar result has often been

noticed in this laboratory when a presumably normal urine has been tested with Fehling's solution.

It is quite likely that this acid may occur more frequently in urine than is suspected, probably only in less quantity than contained in the urine just referred to, and to its presence possibly may be attributed the many peculiar and unsatisfactory reactions so often noticed when testing urine with Fehling's solution. Quite likely, too, in some samples of urine, the acid may be contained in sufficient quantity to produce a reduction with Fehling's solution in such a satisfactory manner as to be mistaken for glucose, and thus many erroneous diagnoses of diabetes mellitus may have occurred.

The urine from which the acid was obtained was of a brownish-red tint, perfectly clear and without sediment. [It responded to all of the copper tests, but not to the bismuth and fermentation tests, and had no influence upon polarized light when examined with the polariscope.]

To isolate the acid the following method was employed:

The urine was treated with half its volume of plumbic tribasic acetate solution,¹ and the resulting voluminous precipitate collected on a filter and washed several times with a mixture of equal parts of alcohol and water. The precipitate was then suspended in warm water and hydrogen sulphide passed through until all the lead was precipitated. After expelling the hydrogen sulphide from the fil-

¹ [Prepared, according to Landolt, by dissolving 192 grammes of lead acetate in 640 c. c. distilled water, and adding 64 grammes yellow oxide of lead. The mixture frequently shaken until only a small white precipitate remains. The precipitate allowed to settle, and the supernatant liquid, having a specific gravity of between 1.23 and 1.24, employed.]

trate by boiling, excess of plumbic carbonate was added, and the liquid was gently boiled several minutes, and then filtered while hot. The filtrate was concentrated on the water bath and then kept in a cool place to allow crystallization to occur. The crystals of the lead salt which separated were washed by decantation with a mixture of equal parts of alcohol and water and recrystallized from hot water. Finally, when sufficiently pure they were dissolved in hot water and the lead precipitated by hydrogen sulphide, filtered, and the filtrate containing the free acid evaporated to dryness at about 70° C. The residue was extracted with ethyl ether, and the latter evaporated spontaneously. Several recrystallizations from ether, the final one from a mixture of ether and water, are necessary to obtain the acid in a fairly pure condition. The crystal mass was pressed between bibulous paper and again recrystallized from water.

The acid thus obtained crystallizes in opaque white tetragonal prisms, melts at 140° C., and sublimes in the same prismatic form, the crystals generally radiating from a centre. It is very soluble in water and in ethyl ether, soluble in absolute alcohol and also in ordinary alcohol, sparingly soluble in chloroform, insoluble in benzol, toluol, and in petroleum ether.

When its solution in ethyl ether is evaporated at a temperature of about 60° C., a slight claret-red tint is produced, which occasionally resolves into spots of purple. This purple substance (somewhat resembling murexide) attaches itself to the crystalline mass, producing a very beautiful appearance. The crystals, including the purple substance, dissolve in water, with a disappearance of the purple coloration.

In the spontaneous evaporation of the aqueous solution of the acid no change of color is noticed.

The acid does not contain sulphur or nitrogen.

The acid is absorbed by animal charcoal. When the urine itself is passed through animal charcoal the filtrate becomes dark claret-red in color, and has lost its reducing property.

Sodium hydrate gives a brownish coloration, beginning at the surface of the liquid (due to absorption of oxygen). Oxyphenic acid gives an almost similar reaction, only that a green coloration is first produced, which is not the case with the other acid. The brownish coloration noticed when the diluted urine containing the acid is added to Fehling's solution, is partly due to the action of the alkali of the Fehling's solution upon the acid.

Picric acid causes no change. Upon the addition of sodium hydrate to the mixture of the acid and picric acid, a brownish coloration is produced, similar to that produced by sodium hydrate alone.

No reduction of the bismuth salt in Böttger's test occurs with the acid.

The acid responds to Trommer's test, as also to Fehling's test.

Argentie nitrate is reduced in the cold by the acid.

The fermentation test fails completely.

Its aqueous solution has no effect upon polarized light.

Upon the addition in turn of a dilute neutral solution of ferric chloride, ammonium hydrate, and acetic acid, the play of colors from green to violet, and then to green as with oxyphenic acid, does not occur. It does not respond to the tests for hydrochinon.

With a dilute neutral solution of ferric chloride a

blue coloration is produced which very soon disappears. From this reaction the acid would appear to be a phenol derivative. It forms lead, barium, and calcium salts.

The lead salt crystallizes in heavy needle-like prisms, melting at 209.5° C. It is soluble in hot water, insoluble in benzol, toluol, petroleum ether, absolute or ordinary alcohol, ethyl ether, and chloroform. It is decomposed when passed through animal charcoal, the acid remaining in the charcoal and the lead coming through with the filtrate as an insoluble white compound.

On account of insufficiency of pure material—acid and lead salt—no ultimate analysis has thus far been made. In a short time I hope to have enough material for that purpose, and then a formula for the acid can be constructed, and more learned regarding its source in the human organism. However, two lead determinations in the lead salt have been made:

0.1466 gramme air-dried lead salt gave 0.0717 gramme PbSO_4 , equivalent to 33.50 per cent. of lead.

0.1314 gramme air-dried lead salt gave 0.0649 gramme PbSO_4 , equivalent to 33.66 per cent. of lead.

Mean percentage of the two determinations, 33.58 per cent. lead, indicating that the acid has probably a high molecular weight.

[The lead salt undoubtedly contains water of crystallization, but the amount has not yet been determined.]

I would suggest for this substance, provisionally, the name glycosuric acid.

